Seroconversion, seroreversion, and serowaffling among participants initiating antiretroviral therapy in Project DETECT

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Background: Terminology

Seroconversion: development of antibodies in serum as result of infection or immunization.

Incomplete seroconversion: absence of complete seroconversion.

Seroreversion: decrease in antibodies to levels below the cutoff of an assay.

Serowaffling: a reactive test result followed by non-reactive then again by a reactive result.

Background

1) Incomplete HIV seroconversion and seroreversion are being identified more frequently than previously recognized, particularly in persons who initiate antiretroviral therapy (ART) during acute HIV infection (AHI).

2) There is increased recognition of false-negative tests in PrEP programs, especially with oral fluid tests and injectable PrEP.

3) Increased availability of home, self-tests has resulted in greater testing of HIV-positive persons already receiving ART.

→ This analysis was undertaken to describe patterns of incomplete seroconversion and seroreversion and serowaffling by specimen and test type in Project DETECT.

Methods (1)
Project DETECT

- Prospective, cross-sectional study to evaluate point-of-care (POC) HIV tests in real-time with unprocessed whole blood (WB) and oral fluid (OF) specimens.
- Participants with discordant results were enrolled into a longitudinal substudy.
- Follow-up continued until:
  - HIV-positive participants: concordant reactive results on all HIV tests or 1 year
  - Participants with false-positives: two sequential concordant nonreactive results
- DETECT visit schedule (in days):

- **See Lauren Violette's presentation today on the Geenius Index C3: Session 5: Emerging Technology, Th 230p**

Methods (2)
HIV tests used in Project DETECT

Device	Manufacturer	Specimen
DPP HIV 1/2 Assay	Chembio Diagnostics System	OF, FS WB, VP WB
OraQuick Advance Rapid HIV 1/2 Antibody Test	Orasure Technologies, Inc	OF, FS WB, VP WB
INSTI HIV-1/HIV-2 Rapid Antibody Test	bioLytical Laboratories, Inc	FS WB, VP WB
Determine HIV 1/2 Ag/Ab Combo	Abbott Laboratories	FS WB, VP WB*
Geenius HIV 1/2 Supplemental Assay	Bio-Rad Laboratories, Inc.	FS WB, VP WB
GS HIV-1/HIV-2 Combo EIA	Bio-Rad Laboratories, Inc.	FS WB, VP WB
RealTime HIV-1	Abbott Laboratories	Individual or pools of 10

OF: oral fluid; FS: fingerstick; WB: whole blood; VP: venipuncture
*Not approved for use on venipuncture whole blood

Methods (3)
Terminology

Complete seroconversion: all POC tests reactive at study censoring.

Incomplete seroreversion: at least one test remained non-reactive.

Seroreversion: sustained regression:

Serowaffling: using the same combo of specimen type and device:

Methods (4)
Statistical Analysis

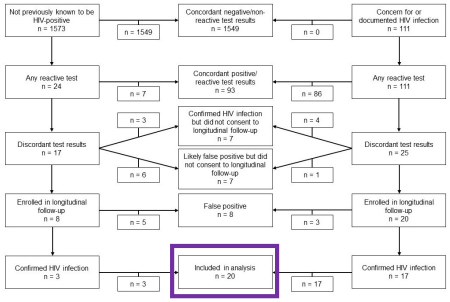
Analysis Plan

- Descriptive: frequencies of seroreversion and serowaffling by participant and specimen type.
- Primary analysis: impact of Fiebig stage at antiretroviral therapy (ART) initiation on incomplete seroconversion, seroreversion, and serowaffling by Fisher's exact tests.
- Dichotomized at stage I-IV versus V-VI because of small numbers.

Methods (5)
Statistical Analysis: Assumptions

- Fiebig stage was determined using clinical and research test results and substituting Geenius when Western blots were not performed.
- In some cases, discrete stages could not be resolved
  Eg. reactive Ag/Ab lab test + nonreactive Geenius = stage II/III
- When testing was not done at ART start, we used the Fiebig stage from the closest visit.

Results (1)
Flow chart of DETECT enrollment and test results



Results (2): Example of complete seroconversion following AHI

Day	Determine VP WB	INSTI VP WB	DPP VP WB	OQ VP WB	Geenius VP WB (GI)	DPP OF	OQ OF	Determine FS WB	INSTI FS WB	DPP FS WB	OQ FS WB	Geenius FS WB (GI)	HIV-1 RNA (copies/mL)	EIA	EIA S/CO[1]
-16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	NR	0.23
-9	ND	ND	ND	ND	Neg	ND	ND	ND	ND	ND	ND	ND	>10,000,000	R	13.21
0	Ab R	R	NR	R	R (0.15)	NR	NR	ND	ND	ND	ND	3,390,000	R	13.21	
2[2]	Ab R	R	R	R	R (0.37)	NR	NR	Ab R	R	R	R	R[3] (0.25)	ND	ND	
7	Ab R	R	R	R	R (1.25)	R	R	Ab R	R	R	R	R[3] (1.05)	29,280	ND	ND

VP: venipuncture; WB: whole blood; GI: Geenius Index; OF: oral fluid; FS: fingerstick; S/CO: signal to cut-off ratio; ND: not done; NR: non-reactive; R: reactive; Ab: antibody
[1]An S/CO ratio ≥1 is considered reactive.
[2]ART start = study day 1.
[3]All specimens were p31 non-reactive.

Results (3): Example of incomplete seroconversion and seroreversion

Day	Determine VP WB	INSTI VP WB	DPP VP WB	OQ VP WB	Geenius VP WB (GI)	DPP OF	OQ OF	Determine FS WB	INSTI FS WB	DPP FS WB	OQ FS WB	Geenius FS WB (GI)	HIV-1 RNA (copies/mL)	EIA	EIA S/CO[1]
-25	ND	ND	ND	ND	NR	ND	ND	ND	ND	ND	ND	ND	4,521,000	R	NA
0[2]	Ab R	R	NR	R	R (0.56)	NR	NR	ND	ND	ND	ND	ND	224	R	13.62
4	Ab R	R	R	R	R (0.72)	NR	NR	Ab R	R	R	R	R[3] (0.24)	ND	R	12.96
7	Ab R	R	R	R	R (0.55)	NR	NR	Ab R	R	R	R	Ind[3] (0.04)	ND	R	13.24
11	Ab R	R	R	R	R (0.18)	NR	NR	Ab R	R	R	R	R[3] (0.20)	ND	R	5.02
13	Ab R	R	R	R	R (0.69)	NR	NR	Ab R	R	R	R	R[3] (0.53)	ND	ND	ND
20	Ab R	R	R	R	R (0.39)	NR	NR	Ab R	R	R	R	R[3] (0.34)	ND	ND	ND
27	Ab R	R	R	R	R (0.39)	NR	NR	Ab R	R	R	R	R[3] (0.31)	ND	ND	ND
41	Ab R	R	R	R	R (0.51)	NR	NR	Ab R	R	NR	R	R[3] (0.22)	ND	ND	ND
115	Ab R	R	R	NR	R (0.16)	NR	NR	Ab R	R	NR	NR	R[3] (0.14)	ND	ND	ND
133	Ab R	NR	NR	NR	R (0.18)	NR	NR	NR	NR	NR	NR	R[3] (0.20)	Undet	ND	ND
174	NR	NR	NR	NR	R (0.13)	NR	NR	NR	NR	NR	NR	R[3] (0.11)	ND	ND	ND
194	NR	NR	NR	NR	Ind (0.04)	NR	NR	NR	NR	NR	NR	R[3] (0.16)	ND	ND	ND
224	NR	NR	NR	NR	Ind (0.03)	NR	NR	NR	NR	NR	NR	R[3] (0.10)	ND	ND	ND
252	NR	NR	NR	NR	Ind (0.05)	NR	NR	NR	NR	NR	NR	NR (0)	ND	ND	ND
286	NR	NR	NR	NR	NR (0)	NR	NR	NR	NR	NR	NR	NR (0)	ND	ND	ND
312	NR	NR	NR	NR	NR (0)	NR	NR	NR	NR	NR	NR	R[3] (0.13)	Undet	ND	ND
340	NR	NR	NR	NR	R (0.07)	NR	NR	NR	NR	NR	NR	R[3] (0.09)	Undet	ND	ND

VP: venipuncture; WB: whole blood; GI: Geenius Index; OF: oral fluid; FS: fingerstick; ; S/CO: signal to cut-off ratio; ND: not done; NR: non-reactive; R: reactive; NA: not available; Ab: antibody; Ind: indeterminate

BLUE= Persistent non-reactive tests
GREEN = Seroreversion
ORANGE = Serowaffling.

[1]An S/CO ratio ≥1 is considered reactive.

[2]PrEP start = d-25
ART start = d-21

[3]All specimens were p31 non-reactive.

Results (4): Example of serowaffling following diagnosis in AHI

Day	Determine VP WB	INSTI VP WB	DPP VP WB	OQ VP WB	Geenius VP WB (GI)	DPP OF	OQ OF	Determine FS WB	INSTI FS WB	DPP FS WB	OQ FS WB	Geenius FS WB (GI)	HIV-1 RNA (copies/mL)	EIA	EIA S/CO[1]	Multispot
-13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	39,480	R	NA	ND
-10[2,3]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	372,200	R	NA	ND
-6[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	108,800	R	NA	ND
0	NR	R	NR	NR	NR (0)	NR	NR	ND	ND	ND	ND	ND	4,919	R	1.66	NR
3	Ab R	NR	NR	NR	Ind (0.05)	NR	NR	NR	NR	NR	NR	R[3] (0.07)	806	R	1.19	NR
7	Ab R	R	NR	NR	Ind (0.03)	NR	NR	NR	NR	NR	NR	NR (0)	ND	NR	0.90	ND
10	Ab R	R	NR	NR	NR (0)	NR	NR	Ab R	R	NR	NR	Ind[3] (0.05)	ND	NR	0.97	ND
14	Ab R	R	NR	NR	R (0.12)	NR	NR	NR	R	NR	NR	Ind[3] (0.05)	49	R	1.28	NR
21	Ab R	R	NR	NR	R (0.16)	NR	NR	Ab R	R	NR	NR	R[3] (0.26)	ND	R	5.64	R
28	Ab R	R	R	NR	R (0.48)	NR	NR	Ab R	R	R	NR	R[3] (0.41)	ND	R	11.01	R
42	Ab R	R	R	R	R (0.79)	NR	NR	R	R	R	R	R[3] (0.44)	ND	ND	ND	ND
56	Ab R	R	R	R	R (0.43)	NR	NR	Ab R	R	R	NR	R[3] (0.51)	ND	ND	ND	ND
70	Ab R	R	R	R	R (0.90)	NR	NR	Ab R	R	NR	R	R[3] (0.49)	Undet	ND	ND	ND
133	Ab R	R	R	R	R (0.34)	NR	R	Ab R	R	R	R	R[3] (0.29)	ND	ND	ND	ND
163	Ab R	R	R	R	R (0.25)	NR	R	Ab R	R	R	R	R[3] (0.51)	ND	ND	ND	ND
193	Ab R	R	R	R	R (0.43)	NR	NR	Ab R	R	R	R	R[3] (0.86)	ND	ND	ND	ND
224	Ab R	R	R	R	R (0.22)	NR	NR	Ab R	R	R	R	R[3] (0.32)	ND	ND	ND	ND
255	Ab R	R	R	NR	R (0.30)	NR	NR	Ab R	R	R	NR	R[3] (0.31)	ND	ND	ND	ND
287	Ab R	R	R	R	R (0.56)	R	NR	Ab R	R	R	R	R[3] (0.40)	ND	ND	ND	ND
316	Ab R	R	R	R	R (0.69)	NR	NR	Ab R	R	R	R	R[3] (0.40)	ND	ND	ND	ND
346	Ab R	R	R	R	R (0.32)	NR	R	Ab R	R	R	R	R[3] (0.23)	ND	R	14.11	ND

VP: venipuncture; WB: whole blood; GI: Geenius Index; OF: oral fluid; FS: fingerstick; ; S/CO: signal to cut-off ratio; ND: not done; NR: non-reactive; R: reactive; NA: not available; Ab: antibody; Ind: indeterminate

GREEN = Seroreversion
ORANGE = Serowaffling.

[1]An S/CO ratio ≥1 is considered reactive.

[2]ART start = day -11

[3]Participant had negative Western Blot assays on these days.

[4]All specimens were p31 non-reactive.

Results (5): Overall outcomes of participants with discordant results

ID[1]	Days from enrollment to diagnosis	Fiebig stage at diagnosis	Days from enrollment to ART start	Fiebig stage at ART start	Complete v. incomplete seroconversion	Seroreversion specimen type	Serowaffling specimen type	Days from enrollment to study censoring[2]
1921	-7	I	10	II/III	Complete			17
1418	-7	II/III	0	IV	Complete			15
1442	-8	II/III	0	IV	Complete			10
1765	-4	II/III	3	IV	Complete			26
1721	-7	II/III	2	V	Complete			6
1644	0	I	37	V	Complete			47
1507	-9	II/III	1	V	Complete			7
1251	0	V	0	V	Complete			11
1582	-2	II/III	0	II/III	Complete		OF/FS	119
1244	-17	II/III	-7	V	Complete		All	33
1927	-12	II/III	2	V	Incomplete[3]			8
1843	0	II/III	n/a	n/a	Incomplete[3]			9
1218	-9	III	0	V	Incomplete	OF[4]		21
1683	-13	II/III	-11	II/III	Incomplete	OF	All	346
1006	-25	II/III	-21	II/III	Incomplete	FS/WB[4]	OF/FS	340
1465	-8	II/III	2	II/III	Incomplete	FS/WB[4]	All	330
1688	-9	II/III	0	IV	Incomplete	OF	OF/FS	210
1378	-20	I	-6	V	Incomplete	OF	OF/FS	366
1815	-16	II/III	1	V	Incomplete	OF[4]	All	347
1175	-1	V/VI	2	VI	Incomplete	OF	All	301

POC: point-of-care; OF: oral fluid; FS: fingerstick; WB: whole blood
[1]These are publication IDs, completely deidentified and no relationship to study ID; to be used across publications.
[2]Follow-up continued until participants had concordant reactive results on all tests, completed one year of follow-up, or were lost to follow-up.
[3]OF remained negative at study censoring at d8 and d9.
[4]One or both OF were persistently negative throughout follow-up.

Results (6) Statistical Analysis

19 (95%) of 20 participants started ART during follow-up
median ART start on day 0 (IQR 0-2, range -21 to 37)

Fiebig Stage at ART start

II/III	IV	V	VI
5	4	9	1

No associations were seen between Fiebig stage at ART start with:
- Complete seroconversion (p=1.0)
- Seroregression (p=.1.0)
- Serowaffling (p=.7)

Limitations

- Small numbers of participants who started ART in AHI
- Assumptions about Fiebig staging at ART start
- We did not follow participants with concordant results to know if they later experienced seroreversion or serowaffling.
- Similarly, it is possible that some participants experienced seroconversion following study censoring.

Conclusions

- There is variability in test performance of different tests on different specimen types.
- Incomplete seroconversion, seroreversion, and serowaffling may represent a growing problem for PrEP programs and HIV testing programs that promote early treatment with hopes of ending the HIV epidemic in the U.S.
- PLWH, especially on ART, should not re-test or be re-tested using POC tests in case non-reactive results lead people to stop ART.
- Additional work is needed to develop and evaluate new testing technologies for HIV screening and diagnosis, particularly with new and upcoming PrEP modalities.

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Study Participants

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